52. BIOCHEMICAL EFFECTS OF TOPICAL APPLICATION AND DECONTAMINATION OF T-2 TOXIN IN RATS

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ABSTRACT

T-2 toxin is a mycotoxin – a natural product of ***Fusarium fungi.*** It was used in the Indochina as a chemical warfare agent and certainly has a "terrorist" dimension. It is very complicated to treat; currently the best antidotes are corticosteroid hormones, compounds with significant adverse effects. These properties make T-2 toxin a very dangerous potential terrorist agent. This research describes our research in decontaminating T-2 on skin. T-2 toxin (1, 5, 50 and 100 μg/cm^2) was administered percutaneously in Wistar rats. In control animals its higher doses exerted progressive topical lesions, ranging from reddening (30 min), oedema and erythema (60 min) to skin necrosis (120 min). They were accompanied by increase in creatine kinase, lactate dehydrogenase, alanine- and aspartate-aminotransferase and decrease in alkaline phosphatase serum activities. Various decontamination agents were used 5, 10, 30, 60 and 120 min after poisoning. Polyethylene glycol 300 (PEG 300) or solution of 5 mg/ml dexamethasone in PEG 300 successfully decontaminated T-2 toxin 50 or 100 μg/cm^2, even if applied 60 min after poisoning. Soap solution 5% was effective only against 1 and 5 μg/cm^2 of T-2 toxin, provided not more than 10 min elapsed after poisoning. Suspension of activated charcoal in water (6:1) and PEG 300 were efficient in rats poisoned with up to 50 μg /cm^2 of T-2 toxin, if applied 10 – 30 min thereafter. When administered topically in control rats, 5% soap solution, activated charcoal and water and solution of dexamethasone in PEG 300 *per se* did not irritate the skin, while PEG 300 caused a mild irritation that lasted 10-12 h. It is concluded that solution of dexamethasone in PEG 300 is the most efficient and safest decontamination agent in rats topically poisoned with T-2 toxin.

INTRODUCTION

The trichothecene mycotoxins are a chemical group of fungal metabolites ⌟ntagonize⌟g⌷ by the tetracyclic 12,13-epoxy-trichotec-9-ene skeleton. There are more than 80 naturally occurring derivatives produced by various species of fungi, such as *Fusarium*, *Myrothecium*, *Trichothecium*, etc (1,2).

Besides their known systemic toxicity for parenchymatous organs (3,4), trichothecenes are also known as skin irritants, causing skin lesions varying from slight reddening to necrosis (5,6). The mechanism underlying this local effect is believed to reside in mediators with a more general cytotoxic effect on the skin (7,8). Histopathological studies showed that T-2 toxin directly affected the epidermis, thus producing apoptosis in basal cells (9). Besides the local effects on skin, T-2 toxin can penetrate the mammalian skin (10-12) and it has strong toxic resorptive effects after topical application (3,13,14).

This study was therefore undertaken to investigate and qualitative aspects of local irritation, serum enzyme activity and efficiency of decontamination by four decontamination formulations: 5 % soap solution, suspension of superactivated charcoal in water, polyethylene glycol 300 (PEG 300) and solution of 5 mg/ml dexamethasone in PEG 300.

METHODS

Chemicals. T-2 toxin was obtained from the Technological-Ecological Centre, Zrenjanin and it was purified at the Military Medical Academy, Belgrade. All the other chemicals of analytical or HPLC grade were purchased from the commercial sources.

Animal experiments. Male Wistar rats (180-220 g) were obtained from the Military Medical Academy, Belgrade, Yugoslavia. The rats were antagonized for at least one week prior to use and received food and tap water *ad libitum.* All the tested substances were administered topically 24 h after the hair was removed from the dorsal skin of each animal by using the electric clippers.

Experimental animals were poisoned topically with 1, 5, 50 and 100 $\mu g/cm^2$ of T-2 toxin and decontaminated at different time intervals thereafter (5, 10, 30, 60, 120 min). All of them were sacrificed 24 h after poisoning and their sera were used for the spectrophotometric determination of creatine kinase (CK), lactate dehydrogenase (LDH), alanine-aminotransferase (ALT), aspartate-aminotransferase (AST) and alkaline phosphatase (ALP).

Data analysis. Statistical significance was determined by means of Student's t-test and Mann-Whitney U-test, and the differences were considered significant when $p < 0.05$.

RESULTS

Since the preliminary results showed that the two smallest doses of T-2 toxin caused a delayed skin irritation and only mild biochemical changes, for further studies of the efficacy of various decontamination regimens only two higher doses were used. They induced very rapid local skin lesions: reddening (30 min), oedema and erythema (60 min) to skin necrosis (120 min). Time-dependency of the biochemical changes, induced by 50 $\mu g/cm^2$ of T-2 toxin indicate that LDH, CK and AST serum activities are significantly increased in comparison with the control animals (Figure 1). These changes became significant even after 2 h following percutaneous contamination. However, since they were much more accentuated in animals sacrificed at 24 h after T-2 toxin, we chose this time as the most appropriate one for the rest of the experiments.

Moreover, preliminary experiments showed that the soap solution 5% was effective only against 1 and 5 $\mu g/cm^2$ of T-2 toxin, provided not more than 10 min elapsed after poisoning, while the suspension of the activated charcoal in water (6:1) and PEG 300 were efficient in rats poisoned with up to 50 $\mu g/cm^2$ of T-2 toxin, only if applied 10 – 30 min thereafter. This is why we chose the first next time interval, i.e. 60 min as a more serious test of the decontamination efficacy. Table 1 contains data on the efficacy of four decontamination formulations in rats percutaneously exposed to T-2 toxin (50 $\mu g/cm^2$). It is obvious that only the 5 mg/ml solution of dexamethasone in PEG 300 afforded protection against the characteristic increase in AST, ALT, CK and LDH and decrease in ALP serum activity.

Since 5 % soap solution, water-activated charcoal suspension (6:1) and PEG 300, when used 60 min after topical skin contamination with T-2 50 $\mu g/cm^2$, were not efficient, we only kept the dexamethasone-PEG 300 combination for the next experiment. This time a doubled dose of T-2 toxin (100 $\mu g/cm^2$) was used (Figure 2). Dexamethasone solution in PEG 300 abolished the changes in AST, ALT and ALP and significantly alleviated those in CK and LDH serum activities. In addition, this decontamination procedure counteracted local skin lesions induced by T-2 toxin. If used under the same circumstances, but 120 min after T-2, this regimen became ineffective.

When administered topically in control rats, 5% soap solution, activated charcoal and water and solution of dexamethasone in PEG 300 *per se* did not irritate the skin, while PEG 300 caused a mild irritation that lasted 10-12 h.

DISCUSSION

These results corroborate the previous reports that T-2 toxin effectively penetrates mammalian skin *in vitro* (10, 11) and *in vivo* (12). The used dose range of T-2 toxin, i.e. 1-100 μg/cm^2 was the same as used in another study in rats (5). Like in our study, soap with water was less efficient than PEG 300 in □ntagonize□g the skin irritation, and necessitated shorter time interval between the contamination with T-2 toxin and decontamination procedure (5). Although in swines superactivated charcoal paste was less efficient than soap in counteracting T-2 toxin-induced skin lesions (6), the results of the present study suggest that the water-charcoal suspension (6:1) was better than 5 % soap solution.

Our by far the most effective decontamination formulation was 5 mg/ml dexamethasone solution in PEG 300. The addition of dexamethasone into the topical formula was based on the well-known efficacy of corticosteroids to block the arachidonic acid cascade (15) and thus decrease the systemic toxicity of T-2 toxin (2,8,16,17). Parenteral injection of corticosteroids, but not of non-steroidal anti-inflammatory drugs (NSAID) □ntagonize the changes in serum enzyme activities (18). A combination of the PEG-induced decreased penetration through the skin and the local and resorptive anti-inflammatory effect of dexamethasone could explain the similar effect obtained in the current study. This formulation could be of great importance, since significant efforts have been performed in order to increase the preparedness of the developed countries for the possible percutaneous contaminations with various chemical and biological agents, including the mycotoxins (19,20).

It could be concluded that, among the formulations tested, 5 mg/ml solution of dexamethasone in PEG 300 is the most efficient and safest decontamination agent in rats topically poisoned with T-2 toxin.

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KEY WORDS
T-2 toxin, topical use, poisoning, decontamination, dexamethasone

FIGURES AND TABLES

Figure 1. Time-dependency of biochemical lesions induced by T-2 toxin (50 μg/cm^2) in rats

0	65,8	23,1	79	240,4	212,5
0,5	67	23,8	77,4	246,13	214,8
1	78,12	26,72	70	273,44	287,57
2	117,34	39,87	57,43	381,28	415,88
24	191,2	49	38,62	414	560

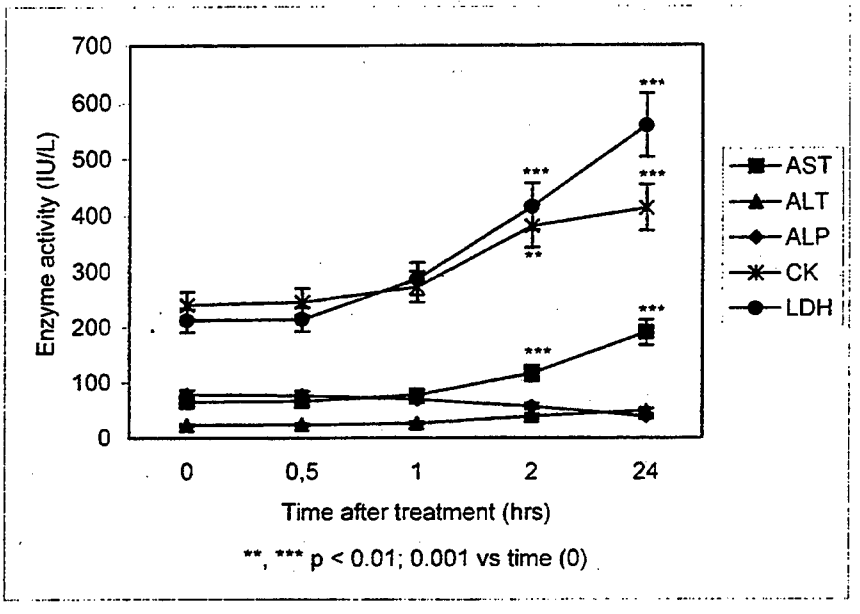

Table 1. The effect of decontamination procedures on serum enzyme activities in rats percutaneously treated with T-2 toxin (50 μg/cm^2)

Treatment	AST	ALT	ALP	CK	LDH
Control	65.8 ± 6.9	23.1 ± 3.1	79.0 ± 8.2	240.4 ± 27.0	212.5 ± 20.6
T-2	136.1 ± 16.9^{a***}	39.1 ± 5.8^{a***}	52.3 ± 8.7^{a***}	341.6 ± 59.6^{a**}	423.1 ± 70.6^{a***}
T-2 + soap solution (5%)	128.0 ± 15.4	33.5 ± 8.6	54.0 ± 9.9	321.0 ± 81.6	409.0 ± 101.2
T-2 + water-charcoal suspension (6:1)	118.7 ± 12.9	32.6 ± 5.9	56.4 ± 8.9	309.9 ± 87.6	398.7 ± 90.3
T-2 + PEG	113.6 ± 20.8	31.2 ± 7.7	59.3 ± 7.9	298.4 ± 65.3	389.7 ± 95.7
T-2 + DXM + PEG	70.5 ± 17.8^{b***}	25.2 ± 8.7^{b**}	74.8 ± 9.5^{b*}	257.3 ± 58.5 ^{b*}	251.8 ± 58.7^{b***}

a*, a**, a*** $p < 0.05$; 0.01; 0.001 vs control group
b*, b**, b*** $p < 0.05$; 0.01; 0.001 vs T-2 group

Figure 2. Antidotal effects of topical dexamethasone in PEG 300 (5 mg/ml) against percutaneous contamination with T-2 toxin (100 μg/cm^2)

121,18	67,43	184,16	102,47
34,08	22,68	147,53	98,18
52,44	76,6	66,37	96,96
402,43	301,2	167,4	125,29
625	288,64	294,11	135,83